

# IMPROVEMENT OF SEEDLING ESTABLISHMENT OF WET SEEDED RICE USING GA<sub>3</sub> AND IBA AS SEED TREATMENT

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## ABSTRACT

Direct seeding has some advantages compared to transplanting system in rice, however there are some constraints need to be addressed. One constraint generally faced in the wet seeded rice is poor stand establishment. The experiment was conducted to improve seedling establishment by using selected growth regulators. Seeds of four rice cultivars, i.e. Memberamo, Widas, MR 84, and MR 219 were treated with GA<sub>3</sub> (gibberellic acid-3) of 25, 50, and 100 mg l<sup>-1</sup>, and IBA (indole-3 butyric acid) of 10, 20, and 40 mg l<sup>-1</sup>. Results showed that cultivar response to GA<sub>3</sub> was similar in all parameters observed, however the response of cultivars to IBA was different at the initial stage. In Indonesian cultivars (Memberamo and Widas), shoot length of IBA treated seedlings was comparable to the control, but the IBA treated seedlings of Malaysian cultivars (MR 84 and MR 219) had slightly shorter shoots compared to the control. GA<sub>3</sub> as seed treatment induced favorable early emergence and shoot growth in the initial stage, but the shoots were etiolated and resulted in susceptible to lodging even in the vegetative stage. In comparison, exogenous application of IBA resulted in high number of adventitious roots contributing towards better establishment of seedling with broader and greener leaves. The growth regulator was lost its effect by 2 or 3 weeks after sowing. All IBA concentrations also did not show any significant differences on growth parameters or have any detrimental effect on grain yield in all cultivar tested. IBA 10 mg l<sup>-1</sup> was sufficient in providing the required improvement in the rice seedlings.

[Keywords: *Oryza sativa*, seed treatment, plant growth substances, direct sowing]

## INTRODUCTION

Direct seeding, one stand establishment technique used in rice, is of interest as a means of reducing cost of production due to less labor requirement (Erguisa *et al.* 1990), shorter rice crop duration (Bangun and Effendi 1993), and more efficient water use (Bhuiyan *et al.* 1995). Although direct seeding has advantages compared to transplanting system, some constraints need to be addressed.

One constraint generally obtained in wet seeded rice is poor stand establishment due to seeds sub-

jected to anaerobic conditions especially if land preparation is not well conducted. The problem becomes more serious when heavy rainfall, infestation by birds and rat occurred at the initial stage of rice establishment which could lead to low plant density, and when plants have bad root system with shallow root distribution (Yamauchi *et al.* 1994). Low plant density will directly reduce yield and also indirectly increase lodging because of reduced mutual support from neighbouring plants.

Establishment of an optimum seedling stand is a critical in crop production, therefore it is important to ensure that seeds emerge early with good root system. One logical approach to improve stand establishment as well as root growth is by using growth regulators as seed treatment. Early emergence and good root system will facilitate plant to get better root anchorage and improve nutrient absorption capability (Kono 1995; Watanabe 1997).

Gibberellic acids-3 (GA<sub>3</sub>) has been known as a growth regulator used to stimulate enzyme production for mobilization of seed reserves in germinating grains and stimulate growth of intact plants (Salisbury and Ross 1992; Arteca 1995). In rice, application of GA<sub>3</sub> as seed treatment significantly improves germination percentage, seedling emergence, and seedling height, especially under suboptimum temperature (Dunand 1992; Bevilacqua *et al.* 1993, 1995; Asborno *et al.* 1999). Indole-3 butyric acid (IBA) is usually used to promote root initiation, adventitious root formation, and early development of root (Weaver 1972; Pan and Zhao 1994; Bellamine *et al.* 1998; Pan and Tian 1999). Generally IBA treated plants have strong and fibrous root system (Weaver, 1972). Although there is some report about the effect of GA<sub>3</sub> and IBA as seed treatment on intact plants, the effect on rice seedling under wet seeded rice is limited. The objective of the present study was to evaluate the effects of GA<sub>3</sub> and IBA as seed treatment on seedling establishment, growth, and yield of wet seeded rice.

## MATERIALS AND METHODS

The experiment was carried out in a screen house at Ladang II Universiti Putra Malaysia (the average of day temperature was  $35^{\circ}\text{C} + 2^{\circ}\text{C}$  and night temperature  $28^{\circ}\text{C} + 2^{\circ}\text{C}$  with daily relative humidity 50-75%). The treatment consisted of two factors. The first factor was rice cultivars, i.e. Memberamo and Widas (Indonesian cultivars), MR 219 and MR 84 (Malaysian cultivars), and the second factor was concentrations of two growth regulators ( $\text{GA}_3$  and IBA). Levels of  $\text{GA}_3$  were 25, 50, and  $100\text{ mg l}^{-1}$ , and IBA were 10, 20, and  $40\text{ mg l}^{-1}$ . The kinds and concentrations of growth regulators used in this experiment were selected based on a preliminary research using four growth regulators at five different concentrations (Wahyuni *et al.* 2003). The experiment was arranged in factorial randomized complete block with four replications.

Each experimental unit comprised of one plastic box (38 cm x 28 cm x 10 cm) and four polyethylene containers (36 cm diameter and 36 cm height). Seeds sown in the plastic boxes were used for destructive harvesting everyday starting from the first to sixth day after sowing (DAS) and 40 seeds were grown in each box. Two containers of each treatment were used for destructive harvesting on the first to third week after sowing (WAS) and the remaining two containers were maintained until harvest for yield analyses. Thirteen pre-germinated seeds were sown in each container (equivalent to  $60\text{ kg ha}^{-1}$ ).

Prior to sowing, the seeds were soaked in growth regulator solution or in distilled water as a control for 48 hours, drained, and then allowed to germinate for 24 hours. The pre-germinated seeds were subsequently sown in the containers filled with 18 kg lowland rice soil obtained from Tanjung Karang, Selangor. The soil was silty clay (fine, mixed, isohyperthermic, Hydraquentic Sulfaquepts) consisting of 39% clay, 42% silt, and 19% sand. The chemical properties of the soil are as follows: 0.15% total N (determined by Kjeldahl method),  $41\text{ mg l}^{-1}$  P-Bray II,  $0.18\text{ me K }100^{-1}\text{ g soil}$ ,  $0.21\text{ me Na }100^{-1}\text{ g soil}$ ,  $3.36\text{ me Ca }100^{-1}\text{ g soil}$ , and  $2.81\text{ me Mg }100^{-1}\text{ g soil}$ .

Water level was gradually increased as seedlings emerged and when seedlings attained 3-4 leaves stage water was maintained at 5-7 cm above soil level. The water level was maintained until one week before harvesting.

Weeding was done manually to eliminate competition. To control insect pests, carbofuran 3%, chlorphirifos 1%, and diazinon  $600\text{ g l}^{-1}$  were applied when required. Fertilizers at  $120\text{ kg N}$ ,  $50\text{ kg P}_2\text{O}_5$ , and

$60\text{ kg K}_2\text{O ha}^{-1}$  were applied as urea, super triple phosphate (TSP), and muriate of potash (KCl), respectively that equivalent to 2.7 g urea, 1 g TSP, and 1 g KCl  $\text{kg}^{-1}$  container. TSP and KCl were applied as basal fertilizer, whereas urea was applied in three equal split applications at 10 and 30 days after rice emergence, and at heading stage (Sharma 1995).

The parameters used to evaluate seedling establishment at the initial stage (1-6 DAS) were shoot length, root length, and number of adventitious roots. Shoot length refers to the distance between plant base (base of coleoptile) and the tip of the highest leaf, and root length is the distance between plant base and the tip of the longest root (Yoshida 1981; Yamauchi *et al.* 1994).

The measurement of shoot and root lengths were continued weekly from the first to third WAS. Shoot and root dry weights were recorded at the same time using standard procedure of AOSA (1981). Leaf chlorophyll content was measured at 1-3 WAS using scanning spectrophotometer type UV 3101 PC. Acetone 80% was used to release chlorophyll from the tissues and the chlorophyll content was calculated based on Coombs *et al.* (1987). Yield components (panicle number per pail and filled grain weight per panicle) and grain yield per container were recorded at harvest time.

The data collected were subjected to analysis of variance using SAS Software Release 6.12 by SAS Institute Inc., USA. Duncan's Multiple Range Test was used to compare treatment means at  $p = 0.05$ .

## RESULTS AND DISCUSSION

### Shoot Growth

The shoot growth of seedlings at the initial stage (0-6 DAS) was dependent on the kinds of growth regulators. In general, shoot emergence was improved by soaking the seeds in  $\text{GA}_3$ . The differences in shoot length were apparent from the third DAS with longer shoot length as  $\text{GA}_3$  concentration increased. Increase in shoot length of  $\text{GA}_3$  treated seedlings were 1.3-2.3 fold compared to the control (Fig. 1). This finding agrees with other studies in which  $\text{GA}_3$  improved emergence of rice seedlings (Dunand 1992; Bevilacqua *et al.* 1993, 1995; Asborn 1999). Exogenous application of  $\text{GA}_3$  enhances the ability of endogenous  $\text{GA}_3$  (Prakash and Prathapasenan 1990) that stimulates  $\alpha$ -amylase activity in germinating seed whereby increases formation of glucose from starch leading to an improved synthesis of sucrose used for growth of seedling (Linn and Kao 1995; Kaur *et al.* 1998).

In comparison, IBA treated seedlings had similar or shorter shoots than control (Fig. 1), possibly due to the inability of IBA in enhancing  $\alpha$ -amylase activity needed in promoting shoot elongation. Kaur *et al.* (1998) showed that auxin (include IBA) was not as effective as  $GA_3$  in enhancing the activity of the enzyme in the cotyledon of chickpea.

All cultivars had a similar response to  $GA_3$  application, although the response was cultivar specific. However, cultivars responded differently to IBA at the initial stage, before 1 WAS, and the differences become narrower in the next stages. Generally, the IBA treated seedlings of Indonesian cultivars (Memberamo and Widas) had taller shoots especially at 4-6 DAS, whereas the Malaysian cultivars were slightly shorter compared to the control (Fig. 1). The differences could be related to their genetic make up or possibly due to the differences in the indigenous  $GA_3$  or IBA content.

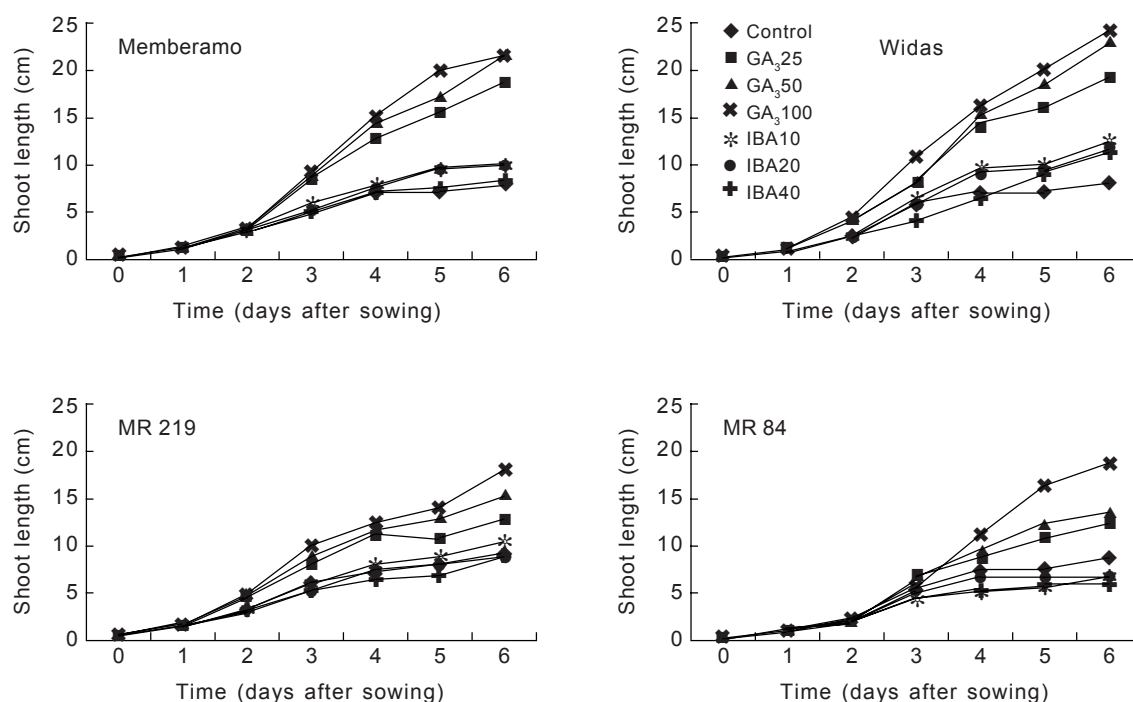
The effect of cultivar on shoot length was significant at 1-3 WAS and the growth regulators was only significant at 1-2 WAS (data not shown). There was an interaction effect between the two factors at 1 and 3 WAS (Table 1). At 1 WAS,  $GA_3$  treated plants at all concentrations maintained longer shoot length compared to the untreated and IBA treated plants in all cultivar tested. The effect of  $100\text{ mg l}^{-1}$   $GA_3$  was still present in seedlings until 2 WAS (data not shown) with seedlings having longer shoots com-

pared to control and IBA treated seedlings. Similar result was reported by Grzesik and Chojnowski (1992) and Kang *et al.* (1993). Gibberellin promoted shoot elongation by increasing plasticity of cell wall

**Table 1.** The interaction effect between growth regulators and cultivars on shoot length at 1 and 3 weeks after sowing (WAS).

Treatment	Shoot length (cm)			
	Memberamo	Widas	MR 219	MR 84
<b>1 WAS</b>				
Control	16.38e	16.83c	18.23b	19.05c
$GA_3$ 25 $\text{mg l}^{-1}$	25.15b	25.08ab	27.78a	25.25b
$GA_3$ 50 $\text{mg l}^{-1}$	31.28a	23.35b	29.53a	28.25ab
$GA_3$ 100 $\text{mg l}^{-1}$	22.60bc	28.28a	29.03a	31.05a
IBA 10 $\text{mg l}^{-1}$	20.73de	17.45c	18.08b	16.35c
IBA 20 $\text{mg l}^{-1}$	18.35de	16.85c	17.75b	18.00c
IBA 40 $\text{mg l}^{-1}$	20.18cde	16.18c	18.23b	16.83c
<b>3 WAS</b>				
Control	51.10ab	48.48	46.95	49.03ab
$GA_3$ 25 $\text{mg l}^{-1}$	50.28ab	47.85	52.85	51.68a
$GA_3$ 50 $\text{mg l}^{-1}$	48.10b	45.93	49.45	51.80a
$GA_3$ 100 $\text{mg l}^{-1}$	48.03b	46.60	48.85	52.18a
IBA 10 $\text{mg l}^{-1}$	49.50ab	48.58	48.03	46.80b
IBA 20 $\text{mg l}^{-1}$	53.05a	49.00	48.45	51.00a
IBA 40 $\text{mg l}^{-1}$	51.60ab	48.05	46.95	49.88ab

Mean values in each column and each time measurement with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT.



**Fig. 1.** The effect of growth regulators on shoot emergence of four rice cultivars.

followed by hydrolysis of starch to sugar which reduces the water potential in the cell resulting in the entry of water into cell causing elongation (Arteca 1995). At 3 WAS, although there was interaction between the two factors as mentioned above, shoot length of all treated plants was comparable to the control in all cultivars (Table 1). It appears that the effect of growth regulators was subsided by time.

Although  $GA_3$  promoted shoot growth, the seedlings were spindly growth with long and narrow leaves, whereas the IBA treated seedlings were non-etiolated, and were greener, healthier and sturdy compared to the  $GA_3$  treated seedlings (Fig. 2). This is supported by the data on leaf chlorophyll content (Tables 2, 3) that the IBA treated plants had higher chlorophyll content compared to those of  $GA_3$ , though it was comparable to the control.

The interaction effect between growth regulators and cultivars on shoot dry weight was only obvious on the first WAS (Table 4). At this stage, untreated seedlings had lower shoot dry weight compared to treated seedlings in Memberamo and MR 219 cultivars, whereas the shoot dry weight of  $GA_3$  and IBA treated seedlings was comparable. There was a compensation effect in term of shoot dry weight between  $GA_3$  treated seedlings which had longer shoots with long and narrow leaves and IBA seedlings which had shorter shoots and broader leaves. Shoot dry weight of treated plants was not significantly different from the control in Widas and MR 84 cultivars.

**Table 2. The interaction effect between growth regulators and cultivars on leaf chlorophyll content at one week after sowing (WAS).**

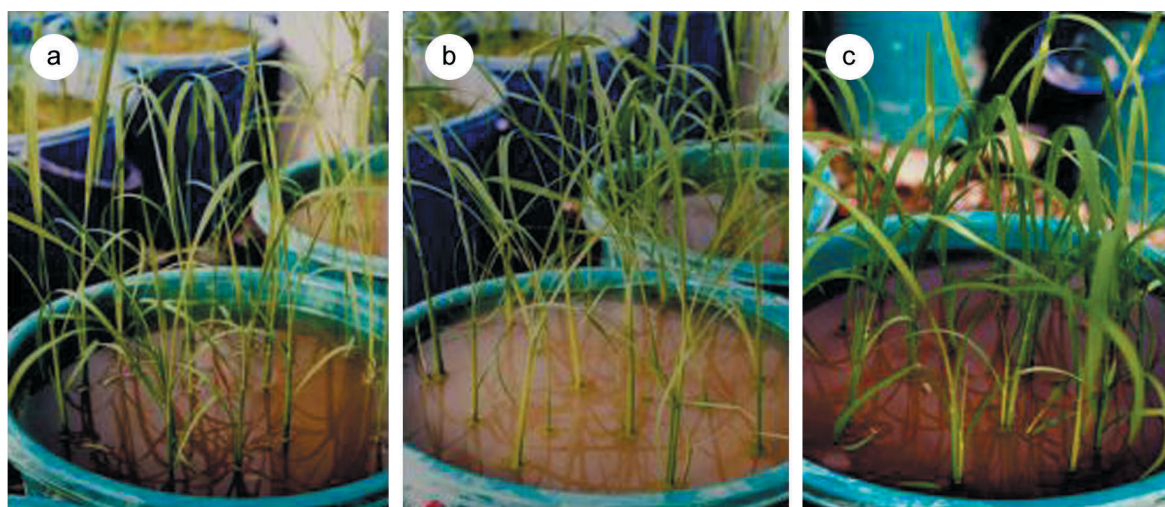
Treatments	Leaf chlorophyll content (mg cm <sup>-2</sup> )			
	Memberamo	Widas	MR 219	MR 84
Control	3.11ab	3.74ab	3.37bc	2.61cd
$GA_3$ 25 mg l <sup>-1</sup>	2.23bc	3.03c	2.88cd	2.12d
$GA_3$ 50 mg l <sup>-1</sup>	2.13c	2.21d	2.41de	2.02d
$GA_3$ 100 mg l <sup>-1</sup>	2.01c	1.73d	1.80e	1.89d
IBA 10 mg l <sup>-1</sup>	3.07ab	3.63ab	3.57ab	3.91a
IBA 20 mg l <sup>-1</sup>	3.54a	3.80a	4.11a	3.52ab
IBA 40 mg l <sup>-1</sup>	3.47a	3.23b	4.08a	3.14bc

Mean values in each column with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT.

### Root Length and Number of Adventitious Roots

In the initial stage,  $GA_3$  stimulated shoot and root elongation, but in the later stage the effect was not obvious. On the other hand, IBA treated seedlings had similar or slightly shorter root length compared to control (data not shown). Similar results were reported by Kang *et al.* (1993) and Kaur *et al.* (1998).

Although there was no effect of IBA on root length, the treatment increased the number of adventitious roots at all observation times (Tables 5, 6). IBA treated seedlings had primary roots with high number of adventitious roots, whereas  $GA_3$  treated



**Fig. 2.** Seedling performance of MR 219 rice cultivar as affected by growth regulators at 2 weeks after sowing; a = control, b =  $GA_3$  100 mg l<sup>-1</sup>, c = IBA 10 mg l<sup>-1</sup>.



**Table 3. The effect of growth regulators and cultivars on leaf chlorophyll content at 2 and 3 weeks after sowing (WAS).**

Treatments	Leaf chlorophyll content (mg cm <sup>-2</sup> )	
	2 WAS	3 WAS
<b>Growth regulator</b>		
Control	3.51ab	3.97ab
GA <sub>3</sub> 25 mg l <sup>-1</sup>	3.23c	3.80b
GA <sub>3</sub> 50 mg l <sup>-1</sup>	3.29bc	3.78b
GA <sub>3</sub> 100 mg l <sup>-1</sup>	3.18c	3.76b
IBA 10 mg l <sup>-1</sup>	3.74a	4.15a
IBA 20 mg l <sup>-1</sup>	3.73a	4.12a
IBA 40 mg l <sup>-1</sup>	3.62a	4.06a
<b>Rice cultivar</b>		
Memberamo	3.27c	3.83a
Widas	3.54ab	3.86a
MR 219	3.72a	3.85a
MR 84	3.36bc	3.95a
<b>Interaction</b>		
Growth regulator x cultivar	ns	ns

Mean values in each column and each factor with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT.

**Table 4. The interaction effect between growth regulators and cultivars on shoot dry weight at one week after sowing.**

Treatments	Shoot dry weight (g)			
	Memberamo	Widas	MR 219	MR 84
Control	11.75b	14.75a	17.75ab	13.00a
GA <sub>3</sub> 25 mg l <sup>-1</sup>	19.75a	17.00a	17.00ab	16.00a
GA <sub>3</sub> 50 mg l <sup>-1</sup>	20.25a	17.00a	18.50a	14.25a
GA <sub>3</sub> 100 mg l <sup>-1</sup>	18.00a	17.50a	15.75c	17.25a
IBA 10 mg l <sup>-1</sup>	18.00a	15.50a	17.25ab	14.25a
IBA 20 mg l <sup>-1</sup>	16.50a	15.00a	18.50a	13.50a
IBA 40 mg l <sup>-1</sup>	17.50a	15.00a	16.50bc	13.00a

Mean values in each column with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT.

**Table 5. The interaction effect between growth regulators and cultivars on number of adventitious roots at 2 weeks after sowing (WAS).**

Treatments	Number of adventitious roots			
	Memberamo	Widas	MR 219	MR 84
Control	2.50bc	3.00bc	0.50a	1.75b
GA <sub>3</sub> 25 mg l <sup>-1</sup>	1.50bc	3.00bc	1.05a	0.75c
GA <sub>3</sub> 50 mg l <sup>-1</sup>	1.50bc	3.50b	1.00a	0.50c
GA <sub>3</sub> 100 mg l <sup>-1</sup>	0.75bc	1.50c	1.00a	0.00d
IBA 10 mg l <sup>-1</sup>	4.00ab	4.00b	1.50a	1.25b
IBA 20 mg l <sup>-1</sup>	5.75a	4.75ab	1.00a	3.00a
IBA 40 mg l <sup>-1</sup>	6.25a	6.25a	1.00a	3.00a

Mean values in each column with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT.

**Table 6. Effect of growth regulator and cultivar on number of adventitious roots.**

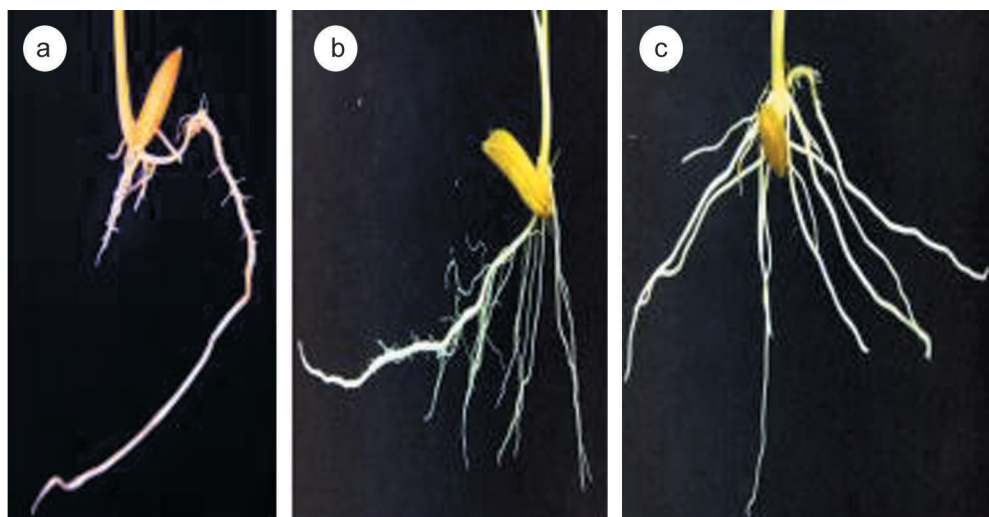
Treatments	Number of adventitious roots			
	3 DAS	4 DAS	5 DAS	6 DAS
<b>Growth regulator</b>				
Control	4.19b	4.88b	5.50b	6.06b
GA <sub>3</sub> 25 mg l <sup>-1</sup>	3.81b	4.88b	5.00b	5.75b
GA <sub>3</sub> 50 mg l <sup>-1</sup>	4.19b	4.88b	5.25b	5.56b
GA <sub>3</sub> 100 mg l <sup>-1</sup>	4.06b	4.81b	5.19b	5.75b
IBA 10 mg l <sup>-1</sup>	6.00a	6.81a	7.56a	7.88a
IBA 20 mg l <sup>-1</sup>	6.38a	7.06a	7.69a	8.56a
IBA 40 mg l <sup>-1</sup>	6.31a	6.63a	7.25a	7.63a
<b>Rice cultivar</b>				
Memberamo	5.14b	6.54a	6.96a	7.61a
Widas	6.57a	7.00a	7.39a	7.82a
MR 219	4.82b	5.36b	5.64b	6.11b
MR 84	3.18c	3.93c	4.82c	5.43b
<b>Interaction</b>				
Growth regulator x cultivar	ns	ns	ns	ns

Mean values in each column and each factor with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT; DAS: days after sowing.

seedlings had primary roots with many root hairs and the control plants only had primary roots with very few adventitious roots (Fig. 3). Early growth of adventitious roots is important especially in direct seeding system. Plants with high number of adventitious roots will presumably have better anchorage in the soil and are able to absorb nutrients. Enhancement of adventitious root number as affected by IBA treatment was also reported by Pan and Zhao (1994), Bellamine *et al.* (1998), and Pan and Tian (1999).

Cultivar differences in number of adventitious roots were significant and Indonesian cultivars (Memberamo and Widas) formed adventitious roots more readily compared to the Malaysian cultivars (Table 6). The differences in number of adventitious roots between cultivars could be related to their genetic make up.

The high number of adventitious roots was reflected in the root dry weight (data not shown). Plant treated with IBA 10 and 20 mg l<sup>-1</sup> had higher root dry weight at 1 WAS. IBA treated plants with better root growth considerably have better root anchorage and are better able to absorb nutrient which to healthy plants with broader and greener leaves.



**Fig. 3.** Effect of growth regulators on root growth of rice cultivar at 5 days after sowing; a = control, b =  $\text{GA}_3$  25 mg  $\text{l}^{-1}$ , c = IBA 10 mg  $\text{l}^{-1}$ .

### Mature Plant

Mature plant development was not affected by seed treatment. Seed treatment with  $\text{GA}_3$  and IBA had no significant effect on mature plant height, tiller number, yield components (data not shown), and yield per container (Table 7). This condition is logical as the effect of exogenously applied  $\text{GA}_3$  and IBA is expected to taper off by 2 or 3 WAS, thus response to growth would ultimately depend on its genetic make up. Table 7 also showed that yield per container was cultivar dependent with MR 219 having the highest yield. Similar result was reported by Dunand (1992).

**Table 7.** Effect of growth regulators and cultivars on yield.

Treatments	Yield per container (g)
<b>Growth regulator</b>	
Control	72.14a
$\text{GA}_3$ 25 mg $\text{l}^{-1}$	73.20a
$\text{GA}_3$ 50 mg $\text{l}^{-1}$	70.36a
$\text{GA}_3$ 100 mg $\text{l}^{-1}$	70.85a
IBA 10 mg $\text{l}^{-1}$	76.84a
IBA 20 mg $\text{l}^{-1}$	73.44a
IBA 40 mg $\text{l}^{-1}$	73.92a
<b>Rice cultivar</b>	
Memberamo	68.75b
Widas	66.34b
MR 219	88.33a
MR 84	68.43b
<b>Interaction</b>	
Growth regulator x rice cultivar	ns

Mean values in each column and each factor with the same letter are not significantly different ( $p = 0.05$ ) based on DMRT; ns: not significant.

Response of seed treatment to panicle number per unit area and grain yield was not significant and was cultivar dependent. Grzesik and Chojnowski (1992) also showed that there was no significant effect of  $\text{GA}_3$  and IBA on both yield and seed quality of *Zinnia elegans*.

Although the effect of seed treatment on yield was not significant, the treatments also did not give any detrimental effect on yield. These were presumably due to the lost effect of growth regulators before plants reached generative stage. Nevertheless, seed treatment with IBA resulted in seedling which had higher number of adventitious roots and greener and broader leaves that contributed toward sturdy and healthy plants (Fig. 1). Therefore, the treatment favored better seedling establishment in the field especially under poor management practices. Since higher concentrations of IBA were not significantly different than 10 mg  $\text{l}^{-1}$  in all parameters observed, application of IBA 10 mg  $\text{l}^{-1}$  was found to be sufficient to provide healthy seedling growth.

### CONCLUSION

Rice cultivars responded differently to IBA treatment at the initial stage, however the effect became similar in the later stage of seedlings. All cultivars had similar response to  $\text{GA}_3$  treatment, although the response was cultivar dependent.  $\text{GA}_3$  improved seedling emergence and shoot growth at the initial stage, but the shoots were elongated and susceptible to lodging, therefore it was not ideal for wet seeded

rice. IBA as seed treatment was better than GA<sub>3</sub> because it improved number of adventitious roots and gave sturdy and healthy seedling with increased leaf chlorophyll content. The treatments did not give any detrimental effect on yield and the difference in yield was cultivar dependent.

The use of IBA as seed treatment has to be considered especially in areas with poor seedling establishment under current practices, or if farmers planting cultivars with bad root system. Application of IBA 10 mg l<sup>-1</sup> was sufficient in providing the required improvement in the rice seedlings.

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